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Developments in . . .

Genetic biodiversity in toxicant-stressed populations

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1 Introduction

The Rio Convention on biodiversity emphasized three levels of biological variability that are threatened by human activities: 1) diversity of ecosystems and landscapes, 2) species richness, and 3) genetic variation within species. The research programmes that have been started in many countries to address the problems raised by the Rio Convention are strongly biased towards issues 1) and 2), while issue 3) is hardly receiving attention. This also holds for ecotoxicology, a science that studies the harmful effects of potentially toxic chemicals on species and communities.

In ecotoxicological research, adverse effects of potentially toxic chemicals are studied in experiments in which designated species are exposed to a graded series of concentrations. Similarly, communities may be exposed to toxicants in outdoor enclosures or microcosms, and toxicological endpoints estimated. Since these experiments aim to support decisions with great financial or societal implications (registration of a pesticide, regulation of certain emissions), it is important that the results are reproducible within reasonable limits. A good deal of attention is therefore given to standardization of species and test conditions. Many ecotoxicological experiments are conducted according to internationally harmonized protocols (see, e.g., Calow, 1993; Løkke and Van Gestel, 1998). This emphasis on standardization is in contrast with the issue of variability evoked by the biodiversity discussion (Van Straalen, 1994). From the perspective of standardization, variability in ecotoxicological experiments is a nuisance, although genetic variation within a species is sometimes considered when test species show interclonal differences in susceptibility to toxicants (Barber *et al.*, 1990; Soares *et al.*, 1992; Crommentuijn *et al.*, 1995).

While genetic biodiversity does not receive much attention in ecotoxicology, it is the main object of study among population geneticists and evolutionary ecologists (Hoffmann and Parsons, 1991). Genetic differences between the individuals of a population form the basis for selection and adaptation processes. The field of

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population genetics has radically changed during the last 10 years owing to the introduction of techniques that may reveal genetic variation directly at the DNA level (Berry *et al.*, 1992). These developments have hardly been taken into account in ecotoxicology, where the traditional emphasis remains at the species level.

There are three different ways in which toxicants may influence genetic variability in a population. First, some toxicants are mutagenic and a high concentration of them in the environment may directly increase the rate of mutation in natural populations. Secondly, toxicants may indirectly increase the mutation rate by affecting DNA repair systems. Thirdly, toxicants may favour those genotypes in a population that are more tolerant than others and so change the genetic composition of the population towards a higher mean tolerance. A higher mutation rate will generally increase genetic variation, while directional selection will normally decrease genetic variation. It is possible that these two opposing forces balance each other; however, given the great variety of chemicals introduced into the environment and the often strong selection pressures exerted by them, exhaustion of genetic variation is often seen as a real risk. This is sometimes referred to as 'genetic erosion': the loss of genetic variation in a population owing to directional selection, drift or inbreeding. It is not known to what extent toxic chemicals may actually have these effects in natural populations. A definitive answer can hardly be given at present, however it is expected that the rapid adoption of molecular methods for measuring genetic variation will provide much more information in the near future. The aim of this paper is to highlight these exciting developments.

II The measurement of genetic variation

One may distinguish three different ways by which the genetic variation in a population may be measured: 1) the biometric approach, 2) the measurement of allozyme polymorphisms and 3) the measurement of DNA polymorphisms.

The biometric approach is derived from animal and plant breeding practice. The animal breeder is concerned with the estimation of the nature and magnitude of differences, phenotypic, genotypic and environmental, between individuals to provide a basis for the selection of individuals to act as parents for the next generation. For selection to be possible there must be phenotypic differences between individuals and, if selection is to result in permanent changes, these differences must be partly genotypic. It is therefore understandable that the science of animal breeding usually deals with quantitative characters such as body size, litter size, milk production, etc., and is particularly interested in estimating the heritability of such characters. Each character is considered as a metric attribute under genetic control by many genes, all making an independent contribution to the phenotypic value of the character.

Heritability is defined as the additive genetic variance of a character relative to the total variance. It is one of the most fundamental concepts in quantitative genetics because it is directly related to the response to selection. This is usually expressed as $R = h^2 S$, where R is the response to selection (the change in the mean value of the character after selection), h^2 is heritability (a number between 0 and 1), and S is the selection differential (the difference between the mean of the selected group and the

mean of the original population). So, the larger the heritability of a character the quicker the population will change under selection.

Heritability may be estimated from experiments in which the metric character is measured on individuals with a known relationship to each other. Posthuma *et al.* (1993) studied heritability of cadmium tolerance in the soil-living collembolan *Orchesella cincta*. Experiments were done using controlled crosses to obtain offspring in which cadmium tolerance (measured by the rate of cadmium excretion) was determined and compared with the tolerance of the parents. Heritability was then estimated from the slope of the parent–offspring regression line. In other experiments, a comparison was made between half-sibs and full sibs. It appeared that heritability of cadmium tolerance was 33–48% in a reference population sampled from a clean area, while there was no significant heritability in another population sampled from a metal-contaminated area. The fact that the tolerant population lacked a significant heritability may be due to an exhaustion of the genetic variation caused by strong directional selection, a result which is in agreement with the 'genetic erosion' hypothesis mentioned above. Like the development of metal-tolerant plant varieties (Verkleij and Schat, 1990), metal tolerance in soil invertebrates provides a nice example of a micro-evolutionary process (Posthuma and Van Straalen, 1993).

The biometric approach is attractive because of its focus on traits with a high ecological relevance (body size, number of offspring, etc.), however the need for a quantitative assessment of many individuals makes this approach very laborious. Estimation of genetic variation from enzyme polymorphisms is more rapid. Many enzymes exist in multiple forms and, when two different varieties are produced by the homologous alleles of one locus in a diploid organism, these polymorphisms may be used to assess genetic variation. Differences between allelic variants often become apparent as a slight variation in molecular charge or structure, with the consequence that the variants behave differently on an electrophoresis gel and may be separated. Within-population variability may be expressed as the fraction of heterozygous loci. When heterozygosity is estimated for a variety of different loci, the average value, which is equivalent to the proportion of heterozygous individuals in the population, may be estimated.

Allozyme polymorphisms to measure effects of environmental contaminants in natural populations have been applied in a number of cases. An example is the study by Kopp *et al.* (1992) on fish populations of sub-basins of the Moose river system in the Adirondack mountains, New York state, USA. A total of 21 loci were investigated in fish from each of seven different basins, with different degrees of acid stress. Eight of the 21 loci investigated were polymorphic among the analysed fish. All five populations in the acidic sites, where total soluble aluminium concentrations varied from 5.6 to 11.4 μM, had a lower heterozygosity than the populations of the control sites which had total soluble Al concentrations of 2.2–2.8 μM. The results strongly suggest that acid stress (possibly correlated with aluminium toxicity) has a negative influence on the genetic diversity of the fish populations. Again, these results support the 'genetic erosion' hypothesis mentioned in the Introduction.

The third way to approach genetic variability is a direct analysis of the DNA of the organisms. In molecular population genetics the polymerase chain reaction (PCR) plays a central role. This technique, used to amplify DNA regions using primers, has tremendously increased the possibilities for application of molecular techniques to the small test animals used in ecotoxicology. DNA fingerprinting can be done without any prior

knowledge of the genome of an organism using PCR with primers of arbitrary sequence (randomly amplified polymorphic DNA, RAPD). In this approach, DNA is extracted from an individual and a polymerase chain reaction is conducted with primers that anneal at unspecified sites of the DNA (Williams *et al.*, 1990). Reaction products are separated by agarose gel electrophoresis and visualized using a dye. Differences between individuals in the basepair sequence of the primer sites will be revealed as different banding patterns. An improvement of the RAPD technique is amplified fragment length polymorphism (AFLP), which uses the PCR after digestion of the DNA and ligation of adaptor sequences that provide a specified template for the PCR primers (Vos *et al.*, 1995).

Another approach in DNA fingerprinting is the analysis of mini- or microsatellites. These are noncoding DNA regions in which a certain sequence, called the 'core sequence', is repeated a number of times, hence these regions are called variable number of tandem repeats (VNTRs). In multilocus fingerprinting the DNA of an organism is cut with restriction enzymes, the fragments are separated on a gel, and a radioactive or fluorescent probe containing the core sequence is used to detect those fragments that have a VNTR. Usually a very complicated array of bands is produced, reminiscent of the 'bar code' used by computers to identify products in a shop. The core sequence in a VNTR is usually 15–60 basepairs long and it may be repeated up to several hundred times. These repeats are widely scattered in the genome, hence this technique provides a multilocus fingerprint. In contrast to minisatellites, microsatellite loci have repeats of only a few (two to ten) units; they are usually employed to develop a single locus fingerprint. Primer sequences are targeted towards the flanking regions of a microsatellite, and a PCR is applied that amplifies a DNA fragment which differs in length between individuals owing to allelic variation in the number of repeated core sequences (Jarne and Lagoda, 1996).

Examples of the application of molecular fingerprinting techniques in an ecotoxicological context are not yet available, but it is expected that in the next few years molecular approaches will invade ecotoxicology, just as they have invaded systematics and population ecology. In research on pesticide resistance, molecular approaches are already very important and they have provided a wealth of new information on the genetic changes that confer resistance to pest insects exposed to plant protection products (McKenzie and Batterham, 1994; Taylor and Feyereisen, 1996).

III Ecological consequences of genetic change

If a natural population is selected by chemical stress, the original genotypes may be replaced by others that are different, not only in tolerance to the stress factor, but also in other aspects. In addition to the general loss of genetic variability illustrated above, there may also be changes correlated with resistance through pleiotropy or genetic linkage. The degree to which selection processes lead to side-effects that must be considered negative is unclear. A generally held opinion among ecologists is that every adaptation bears a cost, because energy allocated towards increased detoxification cannot be spent on other processes such as growth or reproduction. An extensive line of theoretical reasoning has been built upon this supposition (see, e.g., Sibly and Calow, 1989), however the empirical evidence is not very strong. Calow (1991) estimated the

physiological costs of combating chemical stress in animals to be only a few percent of the energy intake at most. In the case of metal tolerance in plants, studies on *Silene vulgaris* suggest that copper tolerance is inherited through a simple mechanism with one major gene, in combination with enhancing factors, determining resistance (Schat and Ten Bookum, 1992). The fact that tolerant plants show a decreased growth rate (with consequent lower competitive capacity than the wild type) seems to be an adaptation to the poor fertility of soils at heavy metals sites and may not have any physiological relation with metal tolerance. These examples show that the relationship between tolerance and fitness costs is not an easy one.

The literature on pesticide resistance provides some examples where fitness costs of increased tolerance are obvious. Resistance against the organophosphate insecticide temephos in mosquitos is associated with amplification of genes coding for esterases. In highly resistant strains 3% of the total protein in the body of a mosquito may consist of detoxification enzyme; this provides an explanation for the fact that these mosquitos have a reduced development time and fertility, correlated with a lower fitness in the absence of the pesticide. A similar situation applies to organophosphate/carbamate-resistant aphids, DDT-resistant houseflies, and diazinon-resistant blowflies (Roush and Daly, 1990). However, there are several other resistances that do not seem to confer any fitness disadvantage, including increased microsomal mono-oxygenase activity in pyrethroid-resistant predatory mites and resistances that are due to a change in the target (altered acetylcholinesterase, conferring resistance to organophosphates and altered GABA-receptor protein, conferring resistance to cyclodienes). It is clear that fitness disadvantages cannot be assumed for every pesticide resistance; they seem to be most clearly associated with mechanisms that rely on an overproduction of general esterases and amplification of the genes coding for these proteins.

An interesting case of negative ecological consequences of genetic change is provided in a study by Doelman *et al.* (1994). These authors isolated bacteria, actinomycetes and fungi from soil and showed that isolates from metal-contaminated soils had a higher resistance to cadmium and zinc. Isolates were subsequently tested for their ability to degrade a series of natural organic compounds, with different degrees of degradability (hydroxybenzoic acid, nicotine acid, pectine, etc.). Results showed that zinc contamination in soil has selected a community of micro-organisms with a decreased metabolic flexibility towards natural substrates. This may have consequences for the composition of organic residues that are formed during microbial degradation of organic matter in soil.

IV Conclusions

The examples above have underlined the importance of genetic biodiversity as an endpoint in ecotoxicological assessments. From the examples reviewed, it appears that environmental stress may significantly reduce the genetic variation in a population. Continued selection by toxicants may also cause secondary changes in the natural ecological performance of communities. It is not known to what extent these mechanisms are compensated by increased mutation rates, but it seems that the possibility of genetic erosion owing to environmental toxicants cannot be ignored. More work, using rapid and highly sensitive molecular approaches for estimating genetic

variability, needs to be done to resolve the question of whether genetic biodiversity is challenged by environmental toxicants.

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